

U.S. Serial No. 10/759,099
AMENDMENT ACCOMPANYING RCE
Docket No. AFIP 03-16

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior listings of claims in the application.

LISTING OF CLAIMS:

1-15. (Canceled)

16. (NEW) A method for detecting an analyte with an immunoliposome-nucleic acid amplification assay, comprising:

 encapsulating a plurality of identical nucleic acid segments within closed shell liposomal bilayers,

 associating receptors to the extravesicular surface of said liposomal bilayers, exposing the receptors to an immobilized target analyte, which binds to the liposomal bilayer associated receptors;

 removing unbound liposomal bilayers;

 lysing the bound liposomal bilayers to release the nucleic acid segments;

 amplifying the nucleic acid segments released from said liposomal bilayers, and

 detecting the amplification products of the nucleic acid segments to quantify the amount of the target analyte.

17. (NEW) The method of claim 16, wherein the target analyte is selected from the group consisting of proteins, nucleic acids, carbohydrates, glycolipids, gangliosides, viruses, bacteria, toxins, chemical warfare agents, explosives, poisons, hormones, cancer-specific soluble biological markers, tumor cell-surface markers, and minor cell components in larger cell populations.

18. (NEW) The method of claim 16, wherein the immunoliposome-nucleic acid amplification assay can be used to spatially localize an analyte within a fresh or fixed tissue section.

19. (NEW) The method of claim 16, wherein the plurality of nucleic acid segments comprises 50 to 1,000 identical nucleic acid segments.

20. (NEW) The method of claim 16, wherein the receptors are selected from the group consisting of monoclonal or polyclonal antibodies, antibody Fab' fragments, gangliosides, glycolipids, soluble proteins, dyes, DNA probes, and RNA probes.

21. (NEW) The method of claim 16, comprising anchoring the receptors to the surface of the liposomal bilayers through covalent attachment to a long-chain-length hydrocarbon having 12 to 24 carbons.

22. (NEW) The method of Claim 21, wherein the long-chain-length hydrocarbon comprises carboxylic acids, amines, thiols, alcohols, aldehydes, nitrates, amides, or halides.

23. (NEW) The method of Claim 16, wherein associating receptors to the extravesicular surface of the liposomal bilayers comprises covalently attaching an antibody to glycolipids or phospholipids.

24. (NEW) The method of Claim 16, wherein associating receptors to the extravesicular surface of the liposomal bilayers comprises electrostatically coupling charged receptors to charged lipids in the liposomal bilayers.

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25. (NEW) The method of Claim 24, comprising electrostatically coupling like charges through divalent cations.

26. (NEW) The method of claim 16, comprising anchoring integral membrane protein receptors to the liposomal bilayers by direct incorporation into the liposomal bilayers.

27. (NEW) The method of claim 16, comprising reducing non-specific binding of the liposomal bilayers on an immobilizing substrate by varying the lipid composition of the liposomal bilayers to alter the size of the liposome, the fluidity of the bilayer, or the polarity and charge of the surface of the liposomal bilayer.

28. (NEW) The method of claim 16, further comprising reducing non-specific binding of the liposomal bilayers on an immobilizing substrate by altering the charge density of the surface of the liposomal bilayer.

29. (NEW) The method of claim 16, further comprising reducing non-specific binding of the liposomal bilayers on an immobilizing substrate by attaching polyethylene glycol to the surface of the liposomal bilayer.

30. (NEW) The method of claim 16, further comprising reducing non-specific binding of the liposomal bilayers on an immobilized substrate by varying the length of a spacer arm used to attach the receptors to the liposomal bilayers.

31. (NEW) The method of claim 16, further comprising reducing background DNA or RNA contamination of the assay by adding DNase or RNase to the assay solution, thereby degrading background DNA or RNA.

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32. (NEW) The method of claim 16, wherein the liposomal bilayers are lysed using a detergent, Triton X-100, an alcohol, or melittin.

33. (NEW) The method of claim 16, wherein said amplifying comprises polymerase chain reaction, real-time PCR, or bDNA or Q-beta replicase methods.

34. (NEW) The method of claim 16, wherein said detecting comprises gel electrophoresis, capillary electrophoresis, or spectrophotometric assays using nucleic acid-specific dyes.

35. (NEW) The method of claim 16, wherein said amplifying and detecting are coupled.

36. (NEW) The method of claim 16, comprising detecting 10 to 1000 molecules of the target analyte.

37. (NEW) The method of claim 16, wherein the target analyte is detected in subattomolar quantities.

38. (NEW) The method of claim 16, further comprising linking a specific receptor to a liposomal bilayer encapsulating nucleic acid segments having a unique nucleotide length, thereby screening for several target analytes at one time.

39. (NEW) The method of claim 16, comprising detecting toxins in soil, water, air, or food.

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40. (NEW) The method of claim 16, comprising detecting contaminants in manufacturing processes or equipment.

41. (NEW) The method of claim 16, comprising detecting target analyte in biological fluids.

42. (NEW) The method of claim 16, wherein the immobilized target analyte is immobilized on a microtiter plate, magnetic micro-particles, or a micro-fabricated device.

43. (NEW) A method for detecting an analyte with an immunoliposome-nucleic acid amplification assay, comprising:

encapsulating a plurality of identical nucleic acid segments within closed shell liposomal bilayers,

incorporating receptors into the outer surface of said liposomal bilayers, wherein the liposomal bilayers become unstable; and

exposing the receptors to a target analyte, thereby causing aggregation of the receptors within the plane of the liposomal bilayers, rupture of the liposomal bilayers, and release of the nucleic acid segments.